FRESH WATER AND MARINE GYMNOSTOMINAN INFUSORIA

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Introduction

The present contribution to the survey of the protozoa deals with the characteristic appearance, habits, and habitats of members of one of the largest and most important groups of the protozoa, namely, the *Gymnostomina*. Within this suborder are included many of the largest of these unicellular forms of animal life; forms which constitute one of the most, if not, indeed, the most important source of food supply for the smaller aquatic organisms, which in their turn form the bulk of the food of fishes. Their presence in ponds and streams is of great importance, for they convert refuse matters which might pollute the water into an available source of food for higher forms of life. A study of the protozoan faunas of waterways should, it seems, go hand in hand with a study of the problems of water purification, and of the preservation and utilization of our aquatic resources.

The majority of the species of *Gymnostomina* treated in this paper are fresh water. Several marine species are also included.

The water samples of which examination was made were secured from various portions of New York, Connecticut, Massachusets, and Mississippi, and over 1,000 were examined. They were taken from open lakes, ponds, roadside pools, rivers, brooks, rills, marshes, watering troughs, and the like.[1] The marine samples were secured from the Connecticut shore of Long Island Sound and from tidal estuaries and embayments, in the vicinity of New Haven.

Methods of Study

Methods of collecting material containing protozoa, in the field, are too well known to need much discussion here. The methods used in the present investigation underwent no decided original modifications from the methods commonly employed.[2]

Half a dozen pint fruit jars, fitted into a small, suit-case-like conveyance, together with a small silk plancton net, a large, long handled cooking spoon, and several glass tubes of various lengths (with detachable compression bulbs for "sucking"), comprised the entire field equipment. The jars were labelled, and a record kept of the nature of the locations from

[1] See Hausman, L. A. Observations on the Ecology of the Protozoa, Am. Nat., vol. 4, 1917, p. 157.

[2] See Hausman, L. A. A Contribution to the Life History of *Amoeba proteus*, Leidy, Biol. Bull., No. 5, May, 1920, p. 340.

which the samples were taken, for future reference. Likewise each precise spot whence samples came was indicated on a topographic map. Possibly this may be found useful at some later time.

Upon arrival at the laboratory the samples were transferred to wide, open-mouthed jars. An examination was made of each sample immediately, and for a week or so, on each succeeding day, with the view of keeping record of the new species which emerged from encystment with the gradual stagnation and putrification of the water, for except in a very few cases the samples contained algae or other vegetal matter. The small bolting silk net shown in Fig. 3 was used for concentrating the infusoria content of one or more pipettefuls of water from the middle or bottoms of the samples where the water was usually more or less clear. No concentration methods were needed in the examination of the surface scum of the putrescing material.

All measurements were made with an ocular micrometer, or from a ruled millimeter slide, from retarded living, or freshly killed specimens.

The characters which are, perhaps, the most satisfactory for use in the identification of the living animals are: the contour of the body, the positions of the buccal cavity and of the largest contractile vacuole, and the disposition of the cilia. Killing and staining, or intra vitam staining may make apparent the structure of the pharynx and also of the nuclear elements. This treatment may sometimes be necessary for bringing out of the cilia. Methods of post mortem and of intra vitam staining will be discussed later.

For the first examination of samples a small drop of water was taken from the top scum, or from concentrated material (the results of straining) and mixed with an equal volume of very viscous gelatine solution, and the whole thoroughly stirred together on the slide with a curved needle.[3] Or often several drops were mixed with an equal part of the gelatine in a watch crystal and used on the slide when needed. The drop on the slide was now carefully flattened out and examined *without a cover glass* under low power (16 mm. objective and 4x eyepiece) to ascertain if the solution were of a viscosity great enough to check sufficiently the movement of the protozoa. If not, it was allowed to concentrate still more by evaporation, until properly viscous, and a cover glass applied. Magnification with the 16 mm. objective and the 4x and 10x eyepieces, and with the 4 mm. objective, and the same two eyepieces was usually found of sufficient strength for the determination of the species described in this paper. A word of caution is to be given here concerning the clarity of the gelatine solution. The gelatine used must be of the best grade and the solution must be

[3] See Hausman, L. A. The Manipulation and Identification of the Free-Swimming Mastigophora of Fresh Waters, Am. Nat., vol. 44, 1920, p. 333.

perfectly fresh. It was found that gelatine which had stood for some time became cloudy in appearance and stringy in texture, due to the growth of colonies of mould plants and bacteria.

Another method of quieting the movements of the protozoa, which was developed, consisted in chilling the slide and its supported water drop on a small block of ice.[3] As the temperature decreased the motions of the protozoa became slower and slower, though never so slow as those incarcerated within the gelatine mixture. This method was devised more in the spirit of curiosity than in any hope that it would be as great an aid as the gelatine method of quieting movement.

Permanent mounts of the infusoria are believed to be very unsatisfactory, with the exception of those made of *Difflugia*, *Arcella*, *Euglypha*, the *Foraminifera*, and others whose bodies secrete a protective shell or test. And here it is the test and not the creature itself which is preserved in its original form. During the process of killing, of staining, and of mounting, the body form is more or less distorted, and the cilia deformed or lost. The most convincing demonstration of the poverty of the mounted slide can be had by examining together a living *Paramoecium* retarded in the gelatine solution, or one freshly stained intra vitam, and a mounted slide, of the same creature, of the best manufacture obtainable. For optimum results in the study of gross anatomy, at least, or for the needs of the systematist, nothing, I think, can equal the intra vitam staining, with the creature hampered in its movements in the gelatine solution. The movements of the cilia or of the contractile vacuoles are often of the greatest aid in determining their position and form. In fact the presence and form of the pharynx in its entire length can often be made out, in certain species, only by means of the cilia vibrating within it.

The stains[4] most frequently used were methyl blue, and gentian violet. Safranin, methyl green, and iodine were also used. Safranin, it was found, stained the deepest, and methyl blue the least. For certain forms, therefore, the one was used, and for others, the other. In the case of each stain a 95% alcoholic solution of the dry stain was made and kept in a small bottle ready to be diluted before applying to the slide. The staining set holder (Fig. 1) was designed to contain in a compact and convenient form the requisite number of stains, and other reagents, together with solid glass dipping rods for each. Thus any mixture of reagents was prevented. The labels (shown underneath the holes for containing the dipping rods) bore the names of the reagents. A great deal of comfort was derived from this very simple piece of apparatus.

The killing and staining was accomplished in two ways: either by killing first and staining afterwards, or by performing both operations simultane-

[4] See formulary of reagents at end of paper.

ously. The killing fluids used were: a 10% aqueous solution of tannic acid, a 1% aqueous solution of copper sulphate, a 2% aqueous solution of osmic acid, a 4% aqueous solution of acetic acid, a 3% aqueous solution of mercuric chloride, a 1% aqueous solution of formaldehyde. The osmic acid and copper sulphate solutions seemed to be the best killers, killing the animals at once, and without apparent distortion. Neither did disintegration set in with such rapidity as was the case when some of the other killing reagents were employed. These killing reagents can be used in other strengths than those given here but these percentages seemed to give the best results.

The killing was done either with a large amount of the material in a watch crystal, or underneath the cover glass, and the staining was accomplished in the same way. Where the protozoa were extremely abundant, as they were usually in surface scums or infusions of decaying marine algae, the watch crystal "mass" staining or killing was found to be the most satisfactory, as well as the easiest and quickest. This method had also to commend it the fact that both the killing reagent and the stain could be most readily controlled. Several watch crystals full of material were placed side by side and very delicate gradations of color secured.

As has been previously stated, the intra vitam staining gave by far the best results. This was accomplished either under the cover glass, or in the watch crystals, following the methods noted above, after the gelatine had been added and the proper degree of viscosity secured.

Preparation of Cultures

In order that a large number of individuals of a given species may be available for examination, it is necessary to depend upon cultures. For convenience in designation, there have been recognized in this paper the following types: (1) natural cultures, that is, those in which large numbers of a species appear, in natural conditions in the field and without any artificial manipulation of the medium in which they occur, (2) indirect cultures, or those which result from merely collecting the material and allowing it to stand and to decompose in the laboratory, and (3) artificial cultures, or those which are prepared with a definite nutritive medium (determined by experimentation) and inoculated with the desired species.

There is little or no exercise of technique involved in securing either natural or indirect cultures. One soons learns to recognize good natural culture environments such as greenish duck ponds, for *Euglenae* of various species; boggy water supporting growths of *Sphagnum*, for *Prorodon niveus* and *armatus;* watering troughs with *Spirogyra* or other *Chlorophyceae*, for species of *Chilodon* and *Holophrya;* clear, cold waters for *Astasia*, etc.

For indirect cultures one has merely to allow the collected water and vegetation to stand in the warmth and light of a south-exposed laboratory window, and make regular examinations day by day.

Where, however, but few individuals of a desired species occur, it becomes necessary to aid their propagation artificially. Results which gave earnest of better ones with further experimentation, were obtained by what is here termed artificial culturing. This was accomplished by segregating desired individuals, and then introducing into the jar of water in which they were placed some favorable nutritive substance. The methodology of preparing such cultures has been well enough developed at the present time, possibly, to make an account worth while, though many problems of detail still await solution.

For capturing individual protozoa under the microscope, there was devised what is here called an *isolation pipette*, shown in Fig. 4. A soft glass tube is drawn out to a hair-like degree of fineness at one end, and inserted into a thin walled rubber tube at the other. The opposite end of the rubber tube is tightly closed by means of a sealed glass tube. The hair-like point of the pipette is first dipped into clear water to allow capillary attraction to draw as much as it will up into the bore. The forefinger of the left hand is laid lightly upon the rubber tube near to its closed extremity, compressing it slightly and thereby driving out a small drop of the water from the tip of the glass pipette. The latter is now inserted with the right hand underneath the objective and into the uncovered drop on the slide. Release of the pressure of the left forefinger results in the withdrawal into the hairlike bore o the pipette a small quantity of water, the amount of which can be delicately regulated.

After the desired animal has been thus captured it is forced out into the water in the isolation jar, and there is added the proper nutritive substance. Thereafter the whole is set in a warm, light place to "ripen." It was found advisable, from the standpoint of ease of handling, to make the culture in small 3 or 4 cm. stender dishes. To inoculate such small cultures it sufficed, on several occasions, to introduce but a single individual. This, however, it must be confessed, was because we could secure no more, and the successes resultant from this meagre inoculation were regarded merely as fortunate accidents.[5]

Several inoculations, aggregating some half dozen, or preferably more, individuals are usually necessary. There was no certain way of determining, save after the anticipated development of the culture, whether the

[5] Single individuals can be removed by means of the isolation pipette, and introduced, for long-continued observations, into a device termed the *micro-aquarium* (See Hausman, L. A., The Vibratile Oral Membranes of *Glaucoma scintillans*, Ehr., Am. Nat., vol. 44, 1920, p. 427.

animals had actually been introduced into the isolation jar and inoculation actually accomplished. Rather clumsy attempts, yet in several instances not unsuccessful ones, were made to make as certain as possible the incarceration of single individuals within the isolation jar by first ejecting the captured animal into a drop of clear water on a slide while under the microscope, noting the presence of the creature, and then washing it off carefully into the material in the isolation jar with a fine stream of clear water.

Tubes of different tip diameters were used, and it was noted that the most success attended the use of the finest of these which it was possible to use for a given species. It is well to give the tip a slight turn when drawing it out, as shown in the figure. This seemed to make it easier to manipulate under the microscope.

It was found practicably impossible to manipulate the pipette and to capture the protozoa under any power greater than that afforded by the use of the 10x eyepiece and the 16 mm. objective. To insure the best results the drop of water must be well flattened out, and first freed from annoying débris.

For maturing the cultures rapidly, and under conditions which could be regulated and tabulated for further referene, a culture oven, such as is illustrated in Figure 2 was devised. A large aquarium jar was equipped with perforated tin shelves hung by copper wires from the upper rim of the jar; heated with a carbon filament lamp, placed on a copper wire platform to prevent it coming into contact with the glass bottom of the jar, and covered with a cardboard cover bearing a thermometer. This had the advantage of furnishing to the stender dish cultures placed on the shelves, at once the requisite amounts of heat and light. The temperature of the interior of the oven was regulated by raising or lowering the cardboard cover, propping it up with little wooden blocks.

Samples were dessicated in this oven by removing the cover and allowing the cultures to remain until they had dried. In this way cultures of *Holophrya*, *Prorodon*, and *Loxophyllum* were kept and resuscitated at pleasure. This method of keeping material by dessication might be a useful one for class requirements. The dried material could be removed from the dishes and placed in labelled envelopes, and filed away in a card catalogue tray. More experimentation along this line might reveal the fact of its being possible to have on file any quantity of protozoa material which could be revivified at will for class room use!

Key to the Families and Subfamilies

I. Protozoa possessing, at some stage of the life cycle, locomotor appendages in the form of cilia, either single or fused into membranes. Macro and micro nucleus present. CLASS INFUSORIA

II. Cilia present during the entire life cycle; buccal cavity and anal orifice normally present; contractile vacuole often connected with an excretory tube system.

SUBCLASS CILIATA

III. Cilia more or less alike in form and distribution over the entire body, having a tendency to lengthen (or in some cases to be present only) on the oral or aboral side. Buccal cilia usually a trifle longer than the others.

ORDER HOLOTRICHIDA

IV. Lacking undulating membranes about the buccal cavity, the latter being closed except during the ingestion of food.

SUBORDER GYMNOSTOMINA

A. Body outline usually oval or extended; neither oral nor aboral sides flattened

Family Enchelinidae

AA. Body outline sometimes oval or extended, more often, however, with oral side either flattened or concave.

B. Buccal cavity terminal or nearly so

Family Trachelinidae

BB. Buccal cavity not terminal

C. Gullet with pronounced curve

Family Enchelyidae

CC. Gullet without pronounced curve

D. Body entirely and evenly ciliated

Subfamily Nassulinae

DD. Cilia longer on, or confined to aboral side

Subfamily Chilodontidae

DDD. Cilia confined to oral side

Subfamily Ervilliinae

KEY TO THE GENERA MENTIONED IN THIS PAPER

A. Body ovoid, ellipsoidal, or almost spherical

B. Body distinctly ovoid or spherical

C. Posterior spinous process present ..... UROTRICHA

CC. Posterior spinous process not present

D. Possessing spiral band of longer cilia ..... PERISPIRA

DD. Not possessing spiral band of longer cilia

E. Cilia restricted to one or two circles about the body

F. One midway circle of cilia present ..... MESODINUM

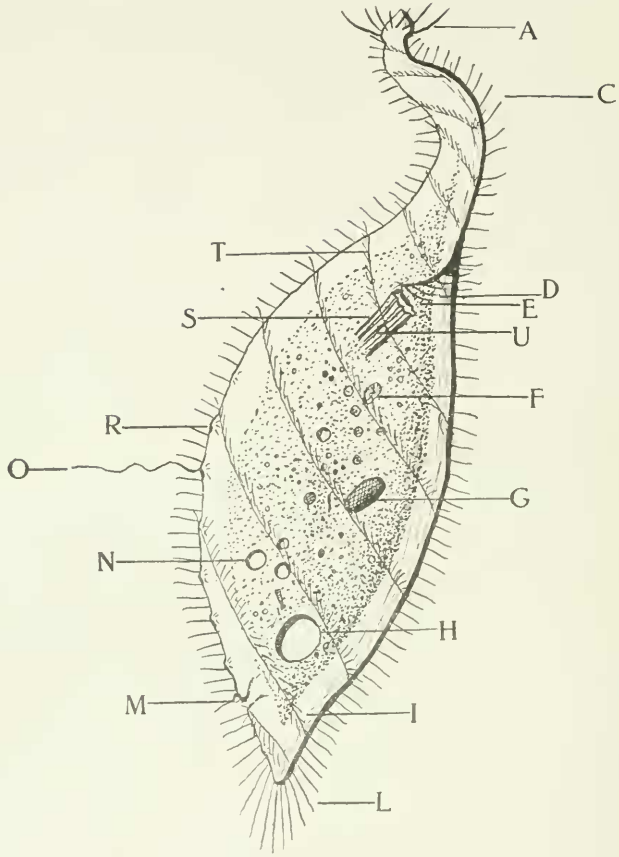
FF. Two such circles present ..... DIDINIUM

EE. Cilia not restricted to one or two circles about the body

F. Cilia restricted to one side of body ..... TROCHILIA

FF. Cilia not restricted to one side of the body

G. Buccal cavity anteriorly terminal
H. Nucleus long and curved.....ENCHELYODON
HH. Nucleus usually ovoidal.....HOLOPHRYA
GG. Buccal cavity not anteriorly terminal
H. With a short neck like, or lip like projection from anterior end.....TRACHELIUS
HH. Without such a projection.....NASSULA
BB. Body not ovoid or nearly spherical, but drawn out into the form of an elongated ellipsoid
C. Body armored with rectangular plates
D. Cilia arising from middle of rectangular plates..... COLEPS
DD. Cilia not arising from middle of plates.....TIARINA
CC. Body not armored with rectangular plates
D. Cilia restricted to ventral surface
E. Body about five times as long as, or longer than, greatest diameter.....LIONOTUS
EE. Body less than five times as long as its greatest diameter .....LIONOTOPSIS
DD. Cilia not restricted to the ventral surface
E. Body longitudinally furrowed.....PLAGIOPOGON
EE. Body not longitudinally furrowed.....PRORODON
AA. Body not ovoid, ellipsoid, or almost spherical
B. Body purse or flask shaped
C. With long flexible neck or proboscis
D. With circle of longer cilia about the anterior extremity of the proboscis.....TRACHELOCERCA
DD. Without such a circle of cilia.....LACRYMARIA
CC. With short neck
D. Neck obliquely truncated
E. Ciliation entire
F. Body capable of distortion at will.....ENCHELYS
FF. Body not capable of distortion.....SPHATHIDIUM
EE. Ciliation not entire.....PHASCOLODON
DD. Neck not obliquely truncated
E. Tentacular process arising from the buccal cavity in the anterior end of neck.....ILEONEMA
EE. Without such a tentacular process TRACHELOPHYLLUM
BB. Body not purse or flask shaped
C. Body ribbon or leaflike
D. Body very elongate
E. Anterior end rounded.....AMPHILEPTUS
EE. Anterior end not rounded.....FLEXIPHYLLUM



An ideal composite gymnostominan ciliate, to show the various anatomical divisions and organs of the body.

a. proboscis cilia
c. proboscis
d. buccal cilia
e. buccal cavity opening into pharynx
f. food vacuoles
g. nucleus
h. principal contractile vacuole
i. hyaline border of body
l. caudal cilia
m. anus
n. smaller contractile vacuole
o. discharged trichocyst
r. undischarged trichocyst
s. pharynx
t. cila band
u. pharyngeal rods

DD. Body not very elongate
E. With anterior border crenulate.....LOXODES
EE. Without crenulations on the anterior border
F. Neck elongated and constricted.....DILEPTUS
FF. Neck not elongated and constricted..LOXOPHYLLUM
CC. Body not leaflike; usually kidney or bean shaped, or nearly so
D. Pharynx long and curved.....TILLINA
DD. Pharynx short and straight.....CHILODON

Observations on the Recorded Species

Genus *Holophrya*

This genus, a very common one, and widely distributed, possesses either an enormous number of distinct species, or a much smaller number, many of which are very variable in size and form, and often, indeed, in coloration. From our limited observations we are inclined to take the latter view. Little attempt has heretofore been made to accord all of these diverse forms specific names.

The species here called Sp. 1 and 6 were commonly found in brackish tidal estuary water about a mile from Long Island Sound among detached, floating *Fucus* and green Sea Lettuce, the first day after having been brought into the laboratory. All of the individuals of these species observed (and there must have been hundreds seen) varied but little away from an average. Later on, however, considerable variation occurred as the numbers became greater in the slowly putrescing material. It is difficult to say whether this was due, however, to an increasing variation among the individual members of the species, or whether new species were making their appearance.

Species 5, 6, and 7 were present in enormous numbers in a four days old infusion of fresh cabbage leaves, and in an infusion of dried corn leaves of the same age.

Species 2, 3, and 4 were met with occasionally in almost all samples, both marine and fresh water, particularly in those from ponds.

The globularity of body, and the small anterior or anterior-lateral buccal cavity, together with the uniform length and distribution of the cilia, appear to be constant characteristics of the members of the genus. Among such small, globular forms, mutilated individuals seemed not to be very common. We are of the opinion that figures of very irregular forms assigned to this genus were made from such mutilated individuals.

Holophrya sp. 1 to 7 shown in Figs. 5-11.

Genus *Urotricha*

Members of *Urotricha* may easily be recognized by the presence of the caudal spine or seta, and by their curious habit of swimming slowly and

evenly and then suddenly jerking ahead, or to the right or left, as though shot by a spring, a motion resulting from a quick lateral snap of the rigid caudal seta.

Kent has observed that the walls of the pharynx are surprisingly elastic, and that this enables the creatures often to take in food, the bulk of which may equal their own bodies!

If the posterior spine is not easily seen, staining makes it easily visible. *Urotricha farcta* and *platystoma*, Figs. 12 and 13.

Genus *Perispira*

The *Perispira ovum* (Fig. 14), of Stein, which we have recorded from stagnant pond waters, may be the same as the *Holophrya ovum*, of Ehrenberg. If this be so then it is an aberrant form of *Holophrya*, for it possesses a spiral band of longer cilia characteristic of *Perispira*. Since even ciliation is characteristic of *Holophyra*, this form had best be placed in *Perispira*.

Genus *Enchelys*

This genus is hardly distinguishable from *Holophrya*, the presence of the laterally opening buccal cavity of *Enchelys* being apparently the sole point of difference. And when the members of the latter assume a globular form, which they do with apparent volition, the buccal cavity becomes almost exactly anteriorly terminal, much like that of many species of *Holophrya*. This assumption of globularity occurs, often, when the animal is gorged with food granules. The young, soon after division has been completed, also take on a globular body.

The smaller species figured is regarded as the *Enchelys farcimen* (Fig. 16) of Ehrenberg. *E. pupa* (Fig. 15) was met with several times in pond water.

Genus *Enchelyodon*

The ovate-elongate body and the terminal buccal cavity, together with the large size, should serve to distinguish *Enchelyodon farctus* (Fig. 17) from forms in the genera *Prorodon* and *Enchelys*. Note that the cilia are very short. We were unable to see them in the unstained animal. This form was rarely found in the waters of bogs, ponds, slowly moving streams, etc.

Genus *Spathidium*

The chief difference between this genus and *Enchelys*, from which it is separated only with difficulty, appears to be the possession of a longer pharynx, usually furnished with pharyngeal rods. The latter, however, are not easily visible.

The species figured, which seems to be *Spathidium spathula* (Fig. 18), was found in pond and slow stream waters.

Genus *Prorodon*

Both *Prorodon armatus* (Fig. 19) and *P. ovum* (Fig. 20) were rarely found in pond waters. The buccal cavities of both are distinct, and the prominent pharyngeal rods of the latter were very good as an identification characteristic.

Genus *Lacrymaria*

Lacrymaria olor (Fig. 21) is a common form in infusions of leaves both of deciduous tress and of aquatic plants. Like *Trachelocerca olor* (Fig. 22) it often lies with its lenticular body concealed among a mass of débris and shoots forth its long serpentine neck in all directions. Whether this is a deliberately willed concealment for the purpose of protection, or for the advantage which it secures for the seizure of prey is uncertain. I have not seen this habit mentioned elsewhere, and yet I found it to be a very common one among the many individuals observed.

Its size is extremely variable, but the constant body form offers a ready means for identification.

Genus *Trachelocerca*

To be distinguished from *Lacrymaria* chiefly by the smaller size of its members. *Trachelocerca olor* (Fig. 22) and *Lacrymaria olor* are almost identical in habits. The movements of the smaller form are, however, the more rapid. *Tracheolocerca olor* is found commonly among the smaller aquatic vegetation in small quiet pools and coves.

Trachelocerca phoenicopterus (Fig. 23) is a marine species occurring among algae along the shore, as well as in putrifying infusions. Its length seems to be very variable.

Genus *Ileonema*

Ileonema dispar (Fig. 24) occurs among *Spirogyra*, *Zygnemea*, *Oscillatoria*, and can probably be found among any of the fresh water filamentous algae. It is not a common form, and usually disappeared soon from fresh material.

The cilia are sparse and apparently weak.

Genus *Plagiopogon*

Plagiopogon coleps (Fig. 25) which we figure from Kent, we believe to have found in salt water among decaying *Fucus* and other algae. It closely resembles *Coleps hirtus* (Fig. 26) though the longitudal furrows of the body and the absence of armor plates are apparent under high powers. It seems to be a species of fairly constant form and size.

Genus *Coleps*

Coleps hirtus (Fig. 26) is a very common form of ciliate, the commonest of its genus, among decaying vegetation and in old infusions, and can be

readily identified from its size and armored body. In swimming it twirls rapidly on its longitudinal axis and pursues a rapid, wavering reckless course. It is an exceedingly voracious species and appears to feed on both animal and vegetable tissue, and the bacteria which are disintegrating them.

Genus *Tiarina*

Tiarina fusus (Fig. 27), a marine form from among decaying algae, resembles *Coleps* in structure very closely. The form of the body is, however, different. The form which we figure we take to be *Tiarina fusus*, (Fig. 27) which is apparently the same as the *Coleps fusus* of Claparède and Lachmann.

Genus *Didinium*[6]

Didinium nasutum (Fig. 28), not an uncommon form in decaying and fresh aquatic vegetation, is one of the largest of the ciliates. Its two zones of cilia offer an easy character for identification. This form appears freely where an adequate supply of smaller ciliates appear, for it is upon these that it feeds. The habits of this species have been exhaustively studied by S. O. Mast (22) and recorded in one of the most interesting of the recent papers on protozoan habits.

The natatory movements of this species are much like those of *Urotricha*, namely a slow gliding progression interrupted frequently by spasmodic jerks.

Genus *Mesodinium*

Mesodinium cinctum (Fig. 30) is not an uncommon form in salt water and when swimming rapidly looks very much like a minute replica of *Didinium nasutum*. The constricted median line and the single zone of median cilia make it easy to identify when at rest.

The smaller *Mesodinium* (Fig. 29), which is found rather rarely associated with the preceding species, I consider to be the *Mesodinium pulex* of Claparède and Lachmann.

Genus *Tillina*[7]

Tillina magna (Fig. 31) were found frequently in a ten day's old infusion of dried corn leaves associated with various species of *Holophrya*, *Chilodon*, and *Colpoda*. Its distinguishing characteristics are the irregular, asymmetrical body and the curved, ciliated pharynx.

Genus *Amphileptus*

Fig. 32 I have called provisionally, *Amphileptus gutta*. It seems to occur in both marine and fresh water infusions. They bear either many smaller contractile vacuoles distributed over the posterior two-thirds of the body,

[6] See Mast, S. O., The Reactions of *Didinium nasutum*, etc., Biol. Bull., vol. 16., 1908, p. 91.

[7] See Gregory, L. H. Observations on the Life History of *Tillina magna*, Jour. Exp. Zool., vol. 6, 1909.

or occasionally, yet not so frequently, one single large vacuole, situated in the posterior half or posterior end, or slightly to one side. Because of a lack of very definite characteristics forms like this are difficult to place with certainty.

Genus *Lionotus*

Members of this genus are among the most graceful ciliates. Viewed from above, the apparently slender neck is seen to be broad and leaflike. Figures of these species should, therefore, indicate this and not lead to the impression that the neck is of the same type as that possessed by *Lacrymaria* or *Trachelocerca*.

Lionotus wrzesniowski (Fig. 36) is a large form, found in pond waters amid living and dead aquatic vegetation, where often occurs, also, *Lionotus fasciola* (Fig. 34). The latter species is also found in salt water with *Fucus* or other marine algae. A similar form, entirely restricted to fresh water, is *Lionotus pleurosigma* (Fig. 35). This species can be distinguished from *fasciola* only by its clear, deep, hyaline border.

The smallest of the species figured (Fig. 33) was found in brackish water in a tidal estuary among detached, floating marine algae.

Genus *Lionotopsis*

Fig. 37 is, perhaps, the *Lionotopsis anser* of Conn, drawn from but a few poorly defined individuals found in pond water. The position of the buccal cavity could not be determined.

Genus *Loxophyllum*

Members of this genus can usually be recognized by the gracefully flexible way in which they glide about over and through débris or wrap their pliant and leaflike bodies about it. The ease with which the curved anterior portion of the body is used for the examination of possible food substances reminds one of the sensitive exploratory gropings of the tip of an elephant's trunk. The deep, clear, hyaline border possessed by all the Loxophylla is constantly characteristic.

Loxophyllum setigerum (Fig. 39) and *rostratum* (Fig. 40) were found quite abundantly in brackish water. The latter appeared in great abundance in an eight days old infusion of green Sea Lettuce and *Fucus* in salt water.

Loxophyllum sp. 1 occurred in fresh water among aquatic vegetation (Fig. 38).

Genus *Trachelophyllum*

Fig. 41 has been called *Trachelophyllum tachyblastum*, from a single specimen found in pond water.

Genus *Flexiphyllum*

Flexiphyllum elongatum (Fig. 42) is frequently met with in pond water among growing vegetation. Its motion is a graceful and sinuous gliding

and it makes rapid progress through the water. We have found that it prefers to move concealed amid débris.

Genus *Trachelius*

Trachelius ovum (Fig. 43) possibly the most common species, can be distinguished by its large size, its curious little neck, and its deliberate motions. The buccal cavity and gullet are quite prominent in most individuals. The size and shape of the neck is apparently subject to considerable variation. Within the body the number of contractile vacuoles is normally very large.

Genus *Dileptus*

Dileptus gigas (Fig. 44), fairly common form, is of unusual variability in size and shape. It is entirely carnivorous and possessed of a voracious appetite. The prey is stung and rendered helpless by the discharge of the trichocysts located along the border of the long neck like process, and if too large to be swept into the buccal cavity by the lashings of the buccal cilia is forced in by the writhings of the neck. The body often rotates on its longitudinal axis during progression through the water.

Individuals have been reported which measured 800 μ.

Genus *Loxodes*

Loxodes rostratum (Fig. 45) was found only rarely in pond water among fresh and decaying vegetation. It is reported to occur also commonly, in infusions.

Genus *Nassula*

This is a beautiful genus, its members being symmetrically ovoid, and many of them iridescent. *Nassula microstoma* (Fig. 48) is a very pretty species. It is usually brownish or yellowish, the color depending upon its contained food. Under strong light, as it revolves through the water, it scintillates brightly, reminding one of a small, ovoid, minutely faceted epidote. This was a very common species in brackish tidal estuary water.

Nasulla ornata (Fig. 46 and *Sp. 1* (Fig. 47) were found in pond waters among fresh and decaying vegetation.

Genus *Chilodon*

Chilodon, much like *Holophrya,* is a genus containing a great number of species of considerable variability of form and size. Of all the species which vary in this way among themselves, *Chilodon cucullulus* (Fig. 52), the commonest, is the most flagrantly disregardful of maintaining its proper dimensions and contour! In the same infusion we have found no less than a dozen differently shaped and sized specimens! Calkins says of this species that it is "extremely variable . . . and has received so many different names that it hardly pays to enumerate them all." It is "one of the most common and widely spread ciliates known."

This species appeared, in a remarkably pure and rich culture, in a five days old infusion of dried flaky deposit from the sides of an old wooden watering trough. It occurred also abundantly in fresh and putrid sea water.

Chilodon megalotrocha (Fig. 49) and *caudatus* (Fig. 51) are sometimes found associated with cucullulus. *Chilodon vorax* (Fig. 50) is much less common.

Genus *Phascolodon*

Phascolodon vorticella (Fig. 53) is a freshwater form from swamps, rather rare. It is a beautiful form, graceful and deliberate in its movements. The body plasm is clear and crystalline.

Genus *Scaphidiodon*

Scaphidiodon sp. 1 (Fig. 54) is perhaps the form shown in Fig. 54. This occurs in sea water containing putrid animal and plant tissues.

Genus *Trochilia*

Trochilia sigmoides (Fig. 55) is another marine form, very striking and beautiful. It is found rarely in clear salt water with living algae, and can be easily recognized by the oblique banding on the body and the meagre number of cilia restricted to one side of the body. Near the small, anterior end of the body is located the buccal cavity, apparently not followed by a definite pharynx, but surrounded by cilia a trifle longer than the rest. It swims slowly, rotating.

FORMULARY OF REAGENTS FOUND USEFUL IN QUIETING, KILLING AND STAINING

I. Quieting Solutions

1. Gelatine solution—Water.....5 oz.
   Gelatine.....1 oz.
   Heat slowly until gelatine is dissolved; then allow to cool and congeal to the desired viscosity.
2. Chlorotone—a 1 per cent aqueous solution

II. Killing Reagents

1. 10 per cent aqueous solution tannic acid
2. 2 per cent aqueous solution osmic acid—Invert slide with drop of water containing the protozoa over a bottle of the solution, uncorked. The fumes kill almost instantly.
3. 3 per cent aqueous solution acetic acid
4. 1 per cent aqueous solution copper sulphate
5. 2 per cent aqueous solution chromic acid

III. Staining Reagents (These can be made up as saturated solutions in either water or 95 per cent alcohol, and diluted to the desired

depths of color. When used in aqueous solutions, very dilute, they make good intra vitam stains.)

1. methyl blue
2. methyl green
3. Lichtgrün
4. Bismark brown
5. Safranin
6. gentian violet
7. iodine, with potassium iodide (a killing stain, either with water or alcohol).

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Explanation of Plate

Fig. 1. Stain or reagent set holder.

Fig. 2. Culture oven *a*, switch, *c*, cardboard cover, *d*, tin shelf, *e*, lamp rack, *f*, copper wire for suspending shelves, *g*, thermometer.

Fig. 3. Strainer for concentrating samples.

Fig. 4. Isolation pipette

Fig. 5. Holophrya sp. 1, 15–25μ

Fig. 6. Holophrya sp. 2, 15–25μ

Fig. 7. Holophrya sp. 3, 15–25μ

Fig. 8. Holophrya sp. 4, 15–25μ

Fig. 9. Holophrya sp. 5, 45–55μ

Fig. 10. Holophrya sp. 6, 30–35μ

Fig. 11. Holophrya sp. 7, 40–45μ.

Fig. 12. Urotricha farcta, 15–25μ

Fig. 13. Urotricha platystoma, 35–45μ

Fig. 14. Perispira ovum, 80–100μ

Fig. 15. Enchelys pupa, 80–100μ

Fig. 16. Enchelys farcimen, 25–50μ

Fig. 17. Enchelyodon farctus, 175–225μ

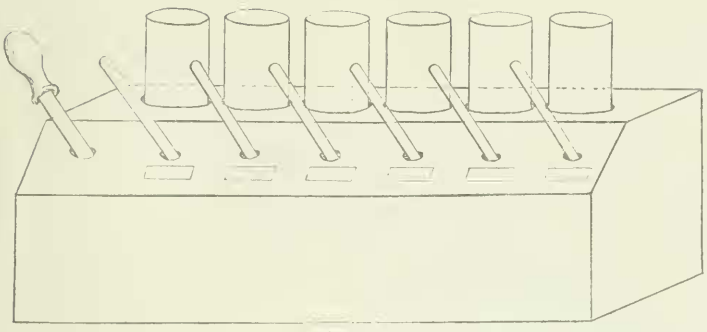
Fig. 18. Spathidium spathula, 60–80μ

Fig. 19. Prorodon armatus, 25–30μ

Fig. 20. (See Plate II)

Fig. 21. (See Plate II)

Fig. 22. Trachelocerca olor, 320–380μ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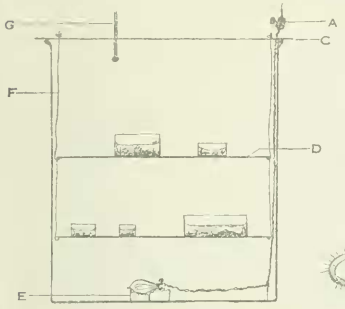

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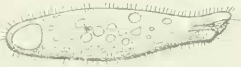
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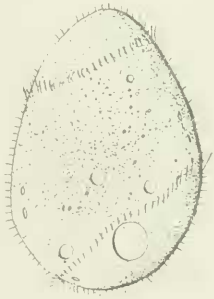
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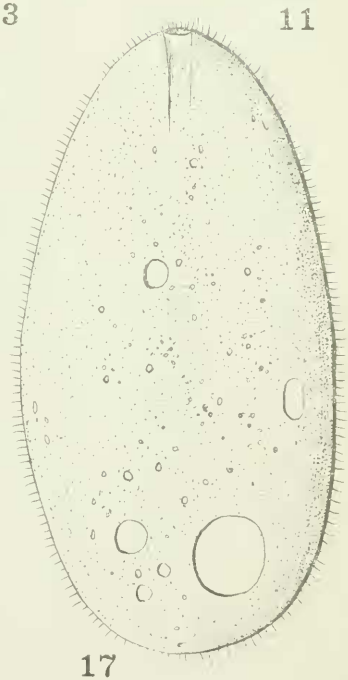
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PLATE V

Explanation of Plate

Fig. 20. Prorodon ovum, 100–130μ.

Fig. 21. Lacrymaria olor, 320–380μ.

Fig. 23. Trachelocerca phoenicopterus, 450–1000μ.

Fig. 24. Ileonema dispar, 115–125μ.

Fig. 25. Plagiopogon coleps, 75–90μ.

Fig. 26. Coleps hirtus, 45–55μ.

Fig. 27. Tiarina fusus, 75–80μ.

Fig. 28. Didinium nasutum, 850–1000μ (the only species not drawn to scale. If represented in its relative proportions, it would be more than twice and a half as large as Trachelius ovum, Fig. 43, Plate IV).

Fig. 29. Mesodinium pulex, 10–20μ.

Fig. 30. Mesodinium cinctum, 30–45μ.

Fig. 31. (See Plate III).

Fig. 32. Amphileptus gutta, 40–60μ.

Fig. 33. Lionotus sp. 1, 25–35μ.

Fig. 34. Lionotus fasciola, 75–125μ.

Fig. 35. Lionotus pleurosigma, 110–125μ.

Fig. 36. Lionotus wrzesniowski, 175–200μ.

Fig. 37. Lionotopsis anser, 75–100μ.

Fig. 38. Loxophyllum, sp. 1, 45–50μ.

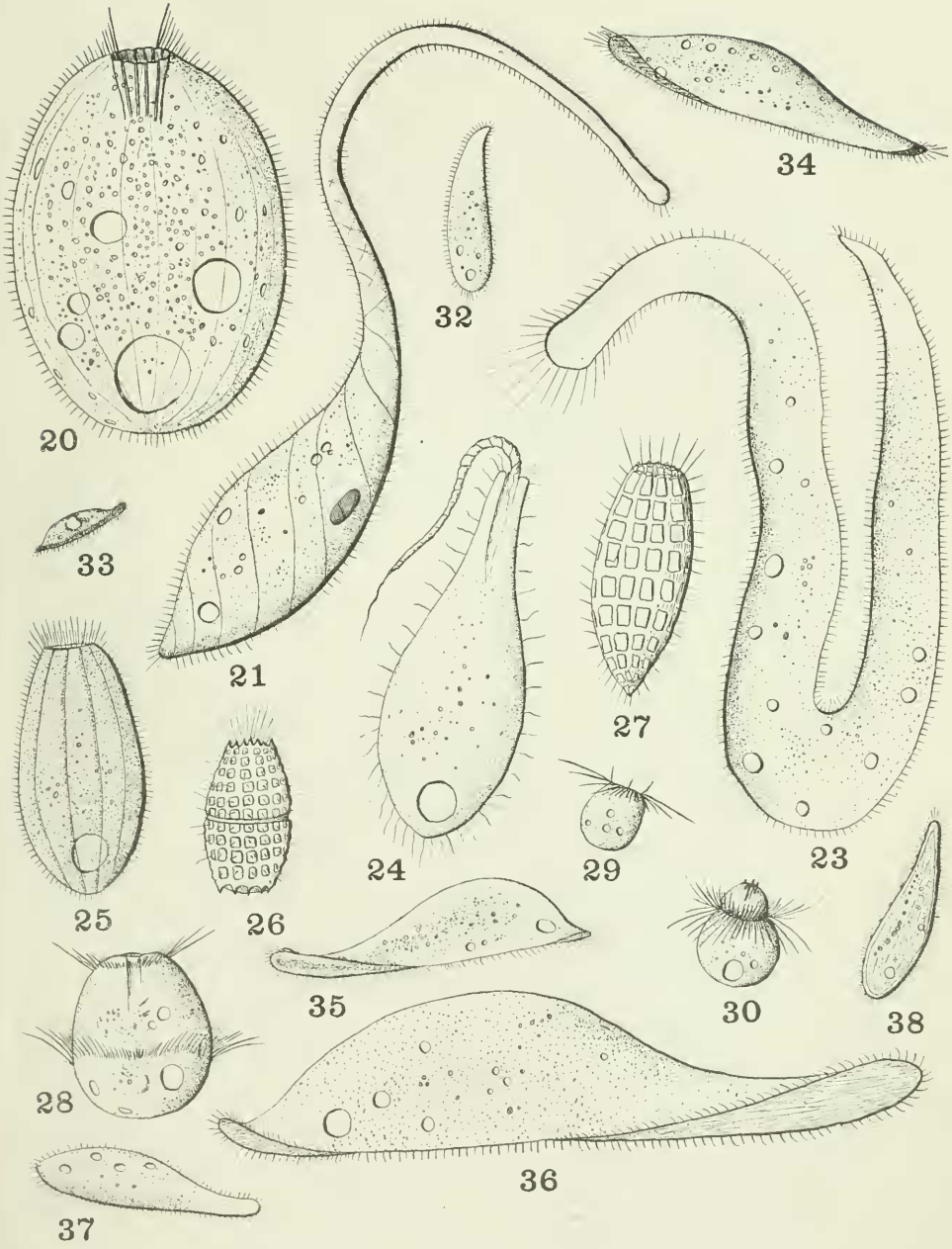

PLATE VI

Explanation of Plate

Fig. 31. Tillina magna, 195–225μ.
Fig. 39. Loxophyllum setigerum, 100–125μ
Fig. 40. Loxophyllum rostratum, 125–150μ
Fig. 41. Trachelophyllum tachyblastum, 120–150μ
Fig. 42. Flexiphyllum elongatum, 200–300μ
Fig. 43. (See Plate IV)
Fig. 44. (See Plate IV)
Fig. 45. Loxodes rostratum, 250–350μ
Fig. 46. Nassula ornata, 200–400μ
Fig. 47. Nassula sp. 1, 30–35μ
Fig. 48. Nassula microstoma, 50–60μ
Fig. 49. Chilodon megalotrocha, 40–50μ
Fig. 50. Chilodon vorax, 50–70μ
Fig. 51. Chilodon caudatus, 35–50μ

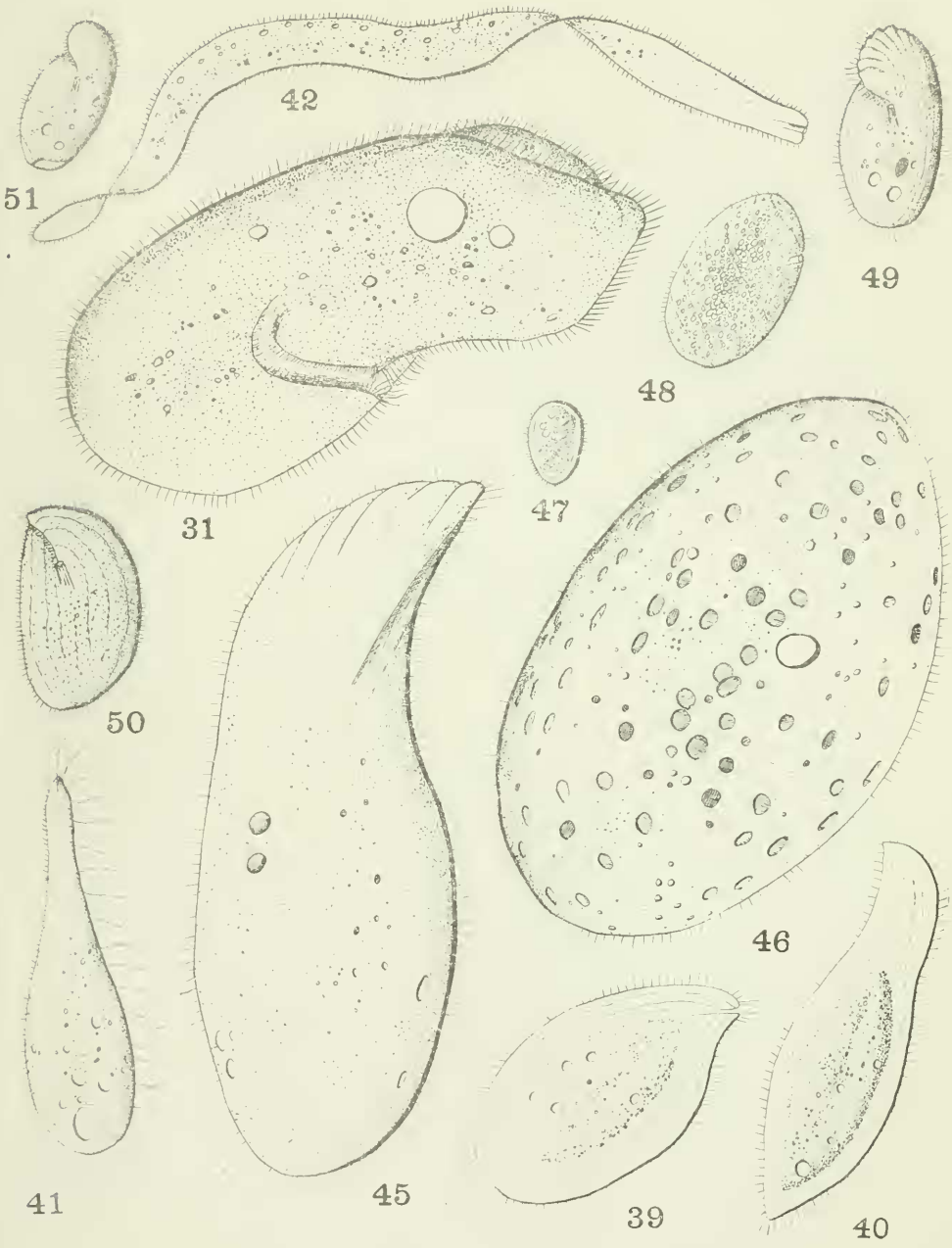

PLATE VII

Explanation of Plate

Fig. 43. Trachelius ovum, 280–350μ
Fig. 44. Dileptus gigas, 450–800μ
Fig. 52. Chilodon cucullulus, 125–225μ
Fig. 53. Phascolodon vorticella, 40–70μ
Fig. 54. Scaphidiodon sp. 1, 25–35μ
Fig. 55. Trochilia sigmoides, 30–40μ

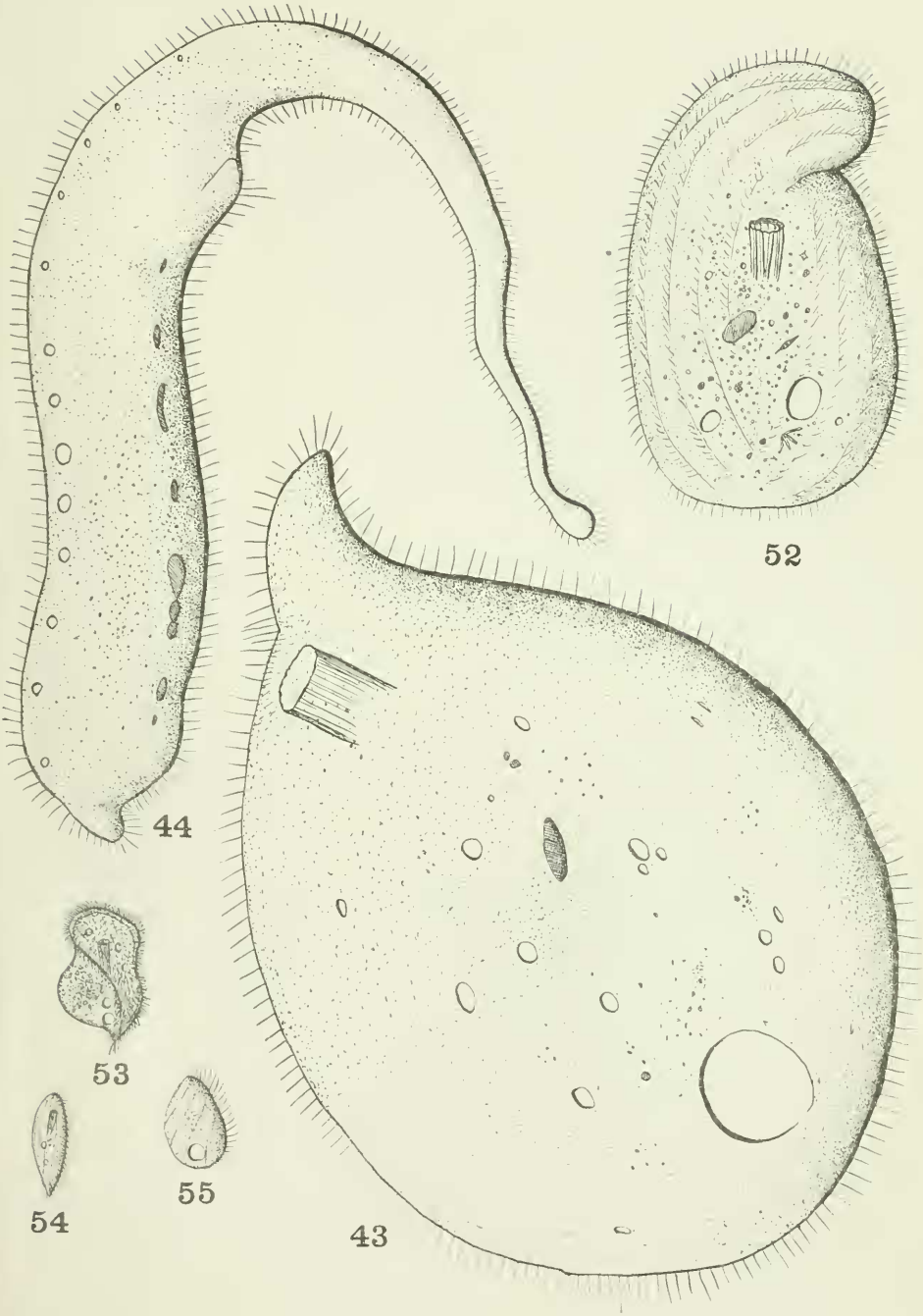

PLATE VIII